Application of yolk immunoglobulin in prophylaxis of diarrhea in piglets

Stefaniak T., Kopeć W. [1], Gąsowska A., Popławski M.[2], Pycio Z.[3]

Department of Vet. Prevention and Immunology, [1] Dept. of Food Technology, Agriculture University of Wrocław, Poland; [2] Swine Farm Łosice; [3] Swine Farm Lutol.
Correspondence: Tadeusz Stefaniak Dept. of Vet. Prevention and Immunology, Agriculture University, ul.Norwida 31, 50-375 Wrocław, Poland, e-mail: tadstef@ozi.ar.wroc.pl ; phone (++4871)3205240, fax (++4871)3730692

Summary The yolk immunoglobulin (IgY) preparate was added to the fodder of piglets to establish its prophylactic role in the periods of highest incidence of diarrhea. IgY was added in the proportion of 0,5g (D1), 1g (D2) or 2g (D3) /kg of the fodder, (control group without IgY) and given to suckling piglets (5-21 days old, in summary 873 from 81 sows at four groups, experiment 1.), or to piglets during first 30 days after weaning (15 piglets in each of the groups, experiment 2.).
Experiment 1.: Diarrhea occurred in 6,72% of suckling control piglets whereas no diarrhea was found in D1 group, in 0,92% of D2, and in 3,21% of D3 group. The incidence of other diseases was also higher in control than in experimental groups. The percent of reared piglets was significantly higher in experimental (89,17 - 91,98%, than in the control (82,21%) groups.
Experiment 2.: No diarrhea or other illness occurred in experimental groups, but one control piglet die from fatal E.coli diarrhea. Experimental piglets showed higher weight gain (from 12 – 15 g /day) than the control.
Keywords: xenogenic passive immunity, yolk antibodies, digestive tract protection, swine

Introduction One of important targets in present pig husbandry is to eliminate antibiotics. Alternative aids are investigated. Results of several groups showed the protection of digestive tract by specific yolk antibodies directed to any diarrheic bacterial and viral strains (Yokoyama et al.1992, 1997, Erhard et al. 1996). Our team elaborated the procedure of production the yolk immunoglobulin on the half-technological scale (patent P-332756). The laboratory results indicate the possible role of specific and cross-reactive yolk antibodies against Gram-negative bacteria.
Aim of the study was to establish the prophylactic role of IgY prepared from non-immunized hen's eggs added to the fodder of piglets in the period of highest incidence of diarrhea.

Materials and methods Animals – Large white polish landrace. Experiment 1. Experimental piglets were fed by the fodder from 5 to 21 day of life. In group D1 – 240 piglets from 22 sows the fodden contained 0,5g IgY/kg, in D2 group – 233 piglets from 22 sows 1 g IgY/kg and in D3 group – 237 piglets from 22 sows – 2 g IgY/kg. Control group 163 piglets from 15 sows were fed without IgY. Experiment 2. Weaned piglets in groups of 15 individuals were fed with the fodder containing 0,5 g IgY/kg (D1), 1 g IgY/kg (D2), 2 g IgY/kg (D3), or without IgY (control group).

Ten trials of yolk immunoglobulin preparations were obtained according to Stefaniak & Kopeć (patent P-332756 / 2000).The preparations were characterized by estimation of the total protein (TP) with biuret method. TP concentration was made on 10g/l and the IgY rate was established by radial immunodiffusion (RID, Mancini). Rabbit anti-chicken IgG serum was raised by immunization, and the RID standard curve was drawn using chicken IgG (Sigma) and pool of 30 hens sera. The yolk imunoglobulin preparations diluted to 0,1g IgY/l were used in the ELISA against alimentary tract pathogens (strains of *Escherichia coli* O157, *Klebsiella pneumoniae, Salmonella Enteritidis, S.typhimurium* obtained from Dr. Grażyna Gościniak, Dept. of Microbiology, Medical University of Wrocław). The bacteria in the concentration of 1 x 10^8/ml of carbonate buffer pH 9,6 were coated on the polystyrene microplates (Organon Teknika). The obtained yolk immunoglobulin reaction was compared to the pooled hens sera and pooled native eggs yolks (Stefaniak & Kopeć 1997).

As the second antibody the rabbit anti-chicken IgG (Sigma) horseradish peroxidase conjugate was used. As substrate the o-phenylenediamine was used, the absorbance was read at 492 nm (Uniscan). Pool of ten characterized preparates was added to the fodder in the proportion of 0,5 g/kg, 1 g/kg, and 2 g/kg according to related experimental groups. 1 g of the fodder was diluted by 10 ml of PBS, mixed, centrifuged and the supernatant reaction against bacterial antigens was tested using ELISA.

Results Table 1 shows the ELISA activity of yolk immunoglobulin preparations and the fodder. The activity of antibodies directed to Gram-negative bacteria differed between preparations tested, but it was present in all of the samples and it was comparable to the activity of hens' sera. No antibody activity was found in control fodder and in the experimental ones the activity increases according to the IgY concentration.

Experiment 1. Piglet were fed with fodder from 5th day of life up to weaning (21st day). Diarrhea occurred in 6,72% of suckling control piglets whereas no diarrhea was found in D1 group. Significantly lower than in control group percent of piglets developed diarrhea D2 group (0,92%), and in D3 group (3,21%). The protection of piglets fed with IgY-containing fodder was seen also against other diseases. Their incidence was also highest in control group (8,92%), and in experimental groups

reached from 8,88% (D1), to 7,41% (D2) and 5,96% (D3). The percent of reared piglets (alive at weaning) was significantly higher experimental groups (89,17%, 92,7% and 91,98% in D1, D2 and D3 respectively, than in the control group (82,21%). No differences were found in the weight gain between control and D2 and D3 groups (0,23 – 0,231kg/day) and only in D1 group it was lower (0,211 kg/day).

Table 1. The intensity of yolk immunoglobulin reaction against selected gram negative bacteria using ELISA.

Material	Absorbance values (λ=492nm)			
	Escherichia coli	Klebsiella Pneumoniae	Salmonella Enteritidis	Salmonella typhimurium
Yolk immunoglobulin preparations (n=10)	0,758 – 1,593	0,32 – 1,152	0,618 – 1,356	0,793 – 1,575
Pooled hens' sera	1,204	0,565	1,288	1,328
Fodder including 0,5 g IgY/kg	0,184	0,216	0,165	0,127
Fodder including 1 g IgY/kg	0,354	0,371	0,374	0,561
Fodder including 2 g IgY/kg	0,763	0,733	0,809	1,014
Control fodder	0	0	0	0

Table 2. Protective influence of IgY added to the fodder of suckling piglets.

Parameter	Percent of piglets			
	Control	D1	D2	D3
Diarrhea	6,72	0	0,93	3,21
Other diseases	8,96	8,88	7,41	5,96
Percent of weaned	82,21	89,17	92,7	91,98
	kg			
Weight gain	0,231	0,211	0,230	0,230

Experiment 2. During 30 days after weaning 15 piglets in each of the groups (D1, D2, D3 and control) were observed. Only in the control group one piglet developed fatal diarrhea, and *E.coli* was isolated from the carcass. No diarrhea or other illness occurred in experimental groups. Piglets from the experimental groups showed higher weight gain (from 12g /day in D2, to 15 g/day in D1 and D3) than the control (Tab.3.). The improvement of group weight gain was over 10% higher in experimental groups than in the control group.

Table 3. Weight gain of weaned piglets protected by IgY added to the fodder.

Groups	Weight gain of the group (kg)	Percent (as 100% control)	Weight gain per day (kg)	Individual rise of the weight during the observation (kg)
Control	105,28	100	0,251	7,52
D1	119,85	113,84	0,266	7,99
D2	118,5	112,56	0,263	7,9
D3	119,7	113,7	0,266	7,98

Discussion It was found that the presence of IgY in the fodder fed to suckling piglets protects them against diarrhea and other diseases, compared to the control group. Significantly more piglets was weaned at 21 days of life when they were fed with the fodder containing yolk immunoglobulin. The results were much better in piglets obtaining 1 or 2 g IgY/kg of the fodder, than in 0,5 g/kg, and the higher doses can be recommended to piglet protection. No influence of the IgY added to the fodder on the improvement of the weight gain was found. It can be associated with higher life rate of piglets from experimental groups, than in control. Moreover it is known, that the weight gain up to 21 day of life is mainly dependent from the quantity of the sow milk.

The obtained results showed also the protective influence of IgY added to the fodder of weaned piglets. Further study on the large scale are necessary to confirm obtained data.

The laboratory results indicate the possible role of specific and cross-reactive yolk antibodies against Gram-negative bacteria.

Conclusion The presence of 1-2g of normal IgY / kg of the fodder improves protection of suckling piglets against diarrhea and other diseases.

Acknowglements The study were supported by grant KBN 5 P06K 033 17.

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